Claims

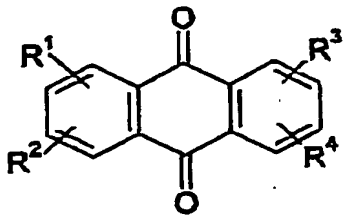
1. A method for detecting the presence of a target nucleic acid sequence in a sample, said method comprising:
(a) adding to a sample suspected of containing said target nucleic acid sequence, a fluorescently labelled probe specific for said target sequence, and a DNA duplex binding agent which can absorb fluorescent energy from the fluorescent label on the probe,
(b) subjecting the thus formed mixture to an amplification reaction in which target nucleic acid is amplified,
(c) subjecting said sample to conditions under which the said probe hybridises to the target sequence, and
(d) monitoring fluorescence from said sample;
said DNA duplex binding agent being one which does not emit visible light during this method.

2. A method according to claim 1 wherein the DNA duplex binding agents has a fused conjugated ring system.

3. A method according to claim 1 or claim 2 wherein the DNA duplex binding agent is mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) or it salt such as the hydrochloride or dihydrochloride salt, nogalamycin (2R-(2α,3β,4α,5β,6α,11β,13α,14α)]-11-[6-deoxy-3-C-mehtyl-2,3,4-tri-O-methyl-α-L-mannopyranosyl)oxy]-4-(dimethylamino)-3,4,5,6,9,11,12,13,14,16-decahydro-3,5,8,10,13-pentahydroxy-6,13-dimethyl-9,16-dioxo-2,6-epoxy-2H-naphthaceno[1,2-b]oxocin-14-carboxylic acid methyl ester) or daunomycin (8S,-cis)-8-acetyl-10-[3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacendione).

4. A method according to claim 3 wherein the DNA binding agent is mitoxantrone.

5. A method according to claim 1 or claim 2 wherein the DNA binding agent is a compound of formula (I)



(IA)

wherein R^1, R^2, R^3 and R^4 are independently selected from hydrogen, X, NH-ANHR and NH-A-N(O)R'R" where X is hydroxy, halo, amino, C_{1-4}alkoxy or C_{2-6}alkanoyloxy, A is a C_{2-4}alkylene group with a chain length between NH and NHR or N(O)R'R" of at least 2 carbon atoms and R, R' and R" are each independently selected from C_{1-4}alkyl and C_{2-4}hydroxyalkyl and C_{2-4}dihydroxyalkyl, provided that a carbon atom attached to a nitrogen atom does not carry a hydroxy group and that no carbon atom is substituted by two hydroxy groups; or R' and R" together are a C_{2-6}alkylene group which, with the nitrogen atom to which R' and R" are attached for a heterocyclic ring having 3 to 7 atoms, with the proviso that at least one of R^1, R^2, R^3 and R^4 is a group NH-A-N(O)R'R".

6. A method according to any one of the preceding claims wherein the target nucleic acid is rendered single stranded prior to hybridisation to the probe in step (c).

7. A method according to any one of the preceding claims wherein the amplification reaction is the polymerase chain reaction (PCR).

8. A method according to any one of the preceding claims wherein the probe hybridises with the target nucleic acid during every cycle of the amplification reaction.

9. A method according to claim 8 wherein the fluorescence from the sample is monitored throughout the amplification reaction.

10. A method according to claim 9 wherein fluorescence data generated is used to determine the rates of probe hybridisation.

11. A method according to any one of claims 8 to 10 wherein the fluorescence data is used to quantitate the amount of target nucleic acid present in the sample.

12. A method according to any one of the preceding claims wherein the fluorescent label is a rhodamine dye, Cy5, fluorescein or a fluorescein derivative.

13. A method according to any one of the preceding claims wherein the fluorescent label is attached at an end region of the probe.

14. A method according to claim 13 wherein the fluorescent label is attached at the 3'end of the probe and prevents extension thereof by a polymerase.

15. A method according to anyone of the preceding claims wherein the probe is designed such that it is released intact from the target sequence during a phase of the amplification process other than the extension phase.

16. A method according to any one of claims 1 to 14 wherein the probe is released intact from the target sequence during the extension phase of the amplification process by the action of the polymerase, and the amplification reaction is effected using a polymerase which lacks 5'-3' exonuclease activity.

17. A method according to claim 1 which comprises performing nucleic acid amplification on a target polynucleotide in the presence of (a) a nucleic acid polymerase (b)at least one primer capable of hybridising to said target polynucleotide, (c) an

oligonucleotide probe which is capable of binding to said target polynucleotide sequence and which contains a fluorescent label and (d) a DNA duplex binding agent which is capable of absorbing fluorescent energy from the said fluorescent label, and which does not emit light in the visible range of the spectrum; and monitoring changes in fluorescence during the amplification reaction.

18. A method according to claim 17 wherein the amplification is suitably carried out using a pair of amplification primers.

19. A method according to claim 17 or claim 18 wherein the nucleic acid polymerase is a thermostable polymerase.

20. A method according to any one of the preceding claims wherein in a further step, a hybridisation assay is carried out and a hybridisation condition which is characteristic of the sequence is measured.

21. A method according to claim 20 wherein the condition is temperature, electrochemical potential, or reaction with an enzyme or chemical.

22. A method according to claim 21 wherein the condition is temperature.

23. A method according to claim 22 which is used to detect allelic variation or a polymorphism in a target sequence.

24. A method for determining a characteristic of a sequence, said method comprising;
a) adding to a sample suspected of containing said sequence, a fluorescently labelled probe specific for said target sequence and a DNA duplex binding agent able to absorb fluorescence from a fluorescent label on the probe but which does not emit radiation in the visible range of the spectrum,

(b) subjecting said sample to conditions under which the said probe hybridises to the target sequence,
(c) monitoring fluorescence from said sample and determining a particular reaction condition, characteristic of said sequence, at which fluorescence changes as a result of the hybridisation of the probe to the sample or destabilisation of the duplex formed between the probe and the target nucleic acid sequence.

25. A method according to claim 24 wherein the reaction condition characteristic of said sequence is temperature, electrochemical potential, or reaction with an enzyme or chemical.

26. A method according to claim 25 wherein the condition is temperature.

27. A method according to any one of claims 24 to 26 wherein the results obtained from two sequences are compared in order to determine the presence of polymorphisms or variations therebetween.

28. A method according to any one of claims 24 to 27 wherein the DNA duplex binding agent is mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) or it salt such as the hydrochloride or dihydrochloride salt, nogalamycin (2R-(2α,3β,4α,5β,6α,11β,13α,14α)]-11-[6-deoxy-3-C-mehtyl-2,3,4-tri-O-methyl-α-L-mannopyranosyl)oxy]-4-(dimethylamino)-3,4,5,6,9,11,12,13,14,16-decahydro-3,5,8,10,13-pentahydroxy-6,13-dimethyl-9,16-dioxo-2,6-epoxy-2H-naphthaceno[1,2-b]oxocin-14-carboxylic acid methyl ester) or daunomycin (8S,-cis)-8-acetyl-10-[3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacendione).

29. A method according to any one of claim 24 to 27, wherein the DNA duplex binding agent is a compound of formula (IA) as defined in claim 5.

30. A kit for use in the method according to any one of the preceding claims, which kit comprises (i) a DNA duplex binding agent which is able to absorb fluorescent energy but which does not emit radiation in the visible range of the spectrum, and either (ii)a fluorescently labelled probe specific for a target nucleotide sequence, or (iii) one or more reagents necessary for conducting an amplification reaction.

31. A kit according to claim 30 which contains (iii) and wherein the reagents are selected from primers, DNA polymerase, buffers, or adjuncts known to improve PCR.

32. A kit according to claim 30 or claim 31 wherein the DNA duplex binding agent is mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) or it salt such as the hydrochloride or dihydrochloride salt, nogalamycin (2R-(2α,3β,4α,5β,6α,11β,13α,14α)]-11-[6-deoxy-3-C-mehtyl-2,3,4-tri-O-methyl-α-L-mannopyranosyl)oxy]-4-(dimethylamino)-3,4,5,6,9,11,12,13,14,16-decahydro-3,5,8,10,13-pentahydroxy-6,13-dimethyl-9,16-dioxo-2,6-epoxy-2H-naphthaceno[1,2-b]oxocin-14-carboxylic acid methyl ester) or daunomycin (8S,-cis)-8-acetyl-10-[3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacendione).

33. A kit according to claim 30 or claim 31 wherein the DNA duplex binding agent is a compound of formula (IA) as defined in claim 5.

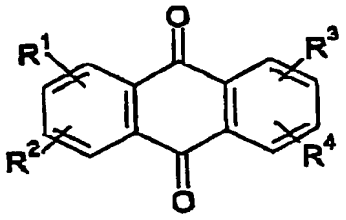
34. A kit according to any one of claims 28 to 33 which comprises both (i) and (ii).

35. The use of a DNA duplex binding agent which can absorb fluorescent energy but which does not emit visible light in a method for detecting the presence of a target nucleic acid sequence in a sample.

36. The use according to claim 35 wherein the DNA duplex binding agent comprises a conjugated aromatic ring system.

37. The use according to claim 36 wherein the DNA duplex binding agent comprises an anthracyclin or anthraquinone.

38. The use according to any one or claims 35 to 37 wherein the DNA duplex binding agent is an optionally substituted anthraquinone of structure (I)



(I)

where R^1, R^2, R^3 and R^4 are independently selected from hydrogen, a functional group, or a hydrocarbyl group optionally substituted by for example functional groups, or R^1 and R^2 or R^3 and R^4 are optionally joined together to form a ring which optionally contains heteroatoms, and/or is optionally substituted by a functional group or a hydrocarbyl group.

39. The use according to any one of claims 35 to 38 wherein the DNA duplex binding agent is mitoxantrone (1,4-dihydroxy 5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione) or it salt such as the hydrochloride or dihydrochloride salt, nogalamycin (2R-(2α,3β,4α,5β,6α,11β,13α,14α)]-11-[6-deoxy-3-C-mehtyl-2,3,4-tri-O-methyl-α-L-mannopyranosyl)oxy]-4-(dimethylamino)-3,4,5,6,9,11,12,13,14,16-decahydro-3,5,8,10,13-pentahydroxy-6,13-dimethyl-9,16-dioxo-2,6-epoxy-2H-naphthaceno[1,2-b]oxocin-14-carboxylic acid methyl ester) or daunomycin (8S,-cis)-8-acetyl-10-[3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacendione).

40. The use according to any one of claims 35 to 38 wherein the DNA duplex binding agent is a compound of formula (IA) as defined in claim 5.

41. The use according to claim 39 wherein the DNA duplex binding agent is mitoxantrone.